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Development of the Platyfish, *Platypoecilus maculatus.*

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(Text-figures 1-25).

Introduction.

The platyfish is a valuable experimental animal being used in research on current problems in physiological and population genetics, chromosomal mechanisms in sex-determination, endocrinology, and in work on the relationship of atypical growth of pigment cells to the general problem of melanoma biology. In all of these problems the pigment cells and both the micro- and macromelanophores of the platyfish play an important part.

The purpose of this paper is to describe a graded series of embryos of *Platypoecilus maculatus* distinguishable on the basis of gross and superficial characters (including pigmentation patterns), and to present data on their developmental rate of growth. Such data may provide a basis of comparison with similar graded series of embryonic stages of the swordtail (*Xiphophorus hellerii*) and with those of their melanotic platyfish-swordtail hybrids. (Gordon, 1931 a & b, 1937).

It was recognized from the beginning that these embryological investigations would be extremely difficult because of the viviparous type of reproduction of the platyfish. Nevertheless, the importance of this species in studies on normal and atypical pigment cell growth has induced us to undertake the project. This paper represents the completion of only the first phase of the problem, namely: a description of a graded series in the early development of the normal, wild-stock platyfish.

The writers are indebted to Dr. Myron Gordon of the New York Aquarium, New York Zoological Society, for his advice and for the use of his facilities and material.

Materials And Methods.

Source and Care of Material.

The fishes used in this work were from a foundation stock of *Platypoecilus maculatus* obtained in 1939 by Dr. Myron Gordon from the Rio Jamapa, Veracruz, Mexico. Most of the strains used belonged to the broods having the New York Zoological Society Culture Numbers 159 and 167. The members of these and related broods have been studied genetically by Gordon (1947).

The adult platyfish, under laboratory conditions, were found to be hardy and adaptable to restricted quarters. Isolated females and mated pairs lived and bred, during the experimental period, up to four months in quart glass jars. Their small aquaria contained conditioned water and were planted with *Nitella* which provided hiding places for the newly born young. The management of mass cultures of platyfish was described by Gordon (1926). The fish were fed daily on a diet that was varied from day to day and consisted of powdered dried shrimp, a fresh liver-Seravim-cereal paste (Gordon, 1943) and living *Daphnia* and *Tubifex*.

The Reproductive Cycle.

The methods used to obtain the various developmental stages depended upon prior knowledge of the reproductive cycle of *Platypoecilus*. For this reason, the details of the cycle are presented below.

Platyfish attained sexual maturity in six to eight months at an average temperature of about 75° F. during the winter months and about 80° F. during the summer. They breed throughout the year and some broods are obtained during every month of the year. These broods are produced at intervals of about 28 days in normal, healthy females.

Young females, shortly after reaching maturity and mating, have broods ranging from one to eight young. The number born increases with the age and size of the mother, averaging 30 to 40 in fully mature fish. In old fish the brood numbers fall off. In a large, wild-caught female, 55 mm. in length, Gordon found 168 embryos, but this was an exception.

Mating and insemination are effected by the male's gonopodium, a sex hormone-induced modification of the anal fin. Females once inseminated and subsequently isolated from further contact with males are capable of producing four or more broods at intervals

[1] Based on material submitted in partial fulfillment of requirements for the Master of Science degree at New York University.

of about 28 days. According to the observations of Gerschler (1914), Winge (1922), and Van Oördt (1929) on *Platypoecilus, Lebistes,* and *Xiphophorus,* sperm which have not fertilized ova do not die but remain viable within the folds of the oviduct for periods up to seven months, in some instances. It is because of this that the ova of isolated females are fertilized as soon as successive complements of ova mature.

Hopper (1943) found that there is an average seven-day interval between the birth of one brood and the fertilization of the next complement of eggs. During this interval, yolk is deposited and maturation takes place. Apparently all the ova do not mature, nor are they fertilized simultaneously. Consequently, the ages of the embryos in any one ovarian sac may vary considerably during early development. Regardless of age variability among the embryos during early development, parturition of an entire brood is accomplished within the space of one hour. During the latter half of the gestation period, then, the belated embryos either catch up to the average morphological age of the brood, or die and become resorbed. The latter possibility is substantiated by the discovery of dead and degenerating embryos after the first few days of the gestation period.

Fertilization of the ova and development of the embryos up to birth, takes place within the ovarian follicle which ruptures just prior to parturition. Toward the end of the gestation period, although the embryos vary somewhat in stages of development, there are no very young embryos in the follicles, nor are there any mature ova. Immediately after the birth of a brood, all the ova present are small and contain little or no yolk. In view of these details, it is clear that superfoetation, which occurs in many poeciliid fishes, is not characteristic of the reproductive behavior of the platyfish.

Collecting Developmental Stages.

In order to obtain a graded series of developmental stages in the young, platyfish virgin females were placed in aquaria containing males. Some females produced their first brood within the month while others did not have theirs for ninety days. After a birth of a brood, the females which were presumed to be gravid again were sacrificed at intervals of from 8 to 25 days and dissected. A total of 63 platyfish was examined and of these 55 carried embryos in various stages of development. The embryos, which were normally retained in the ovarian follicles until birth, were removed from the follicular membrane. They were first examined in 0.9% saline solution and later were preserved in 10% formalin. Counts were made of the number and particular developmental stages of the embryos within each female.

The drawings of the various embryological stages were made from living and preserved specimens. The measurements of the stages are of the total lengths of the embryos, uncurled from their position around the yolk sac.

Description of Normal Development Stages.

Stage 1. *Mature Ovum* (Text-fig. 1).

The mature infertile ova, after the yolk has been deposited, average 1.5 mm. in diameter. They are of a clear yellow color with peripherally arranged fat globules of various sizes. These globules vary somewhat in size and number depending on the individual ovary. However, the eggs in any one ovary are all very similar in this character. When the egg is damaged, the globules are found to be adherent to the peripheral membrane; they are composed of a colorless fluid somewhat less viscous than the yellow colored matrix.

The germinal vesicle cannot be seen in the living egg, but it has been demonstrated by Hopper (1943) to be peripheral in position in sectioned material.

The vitelline membrane is probably present since a fertilization membrane is subsequently demonstrated. No tertiary membrane, such as is found in oviparous species, is present around the egg.

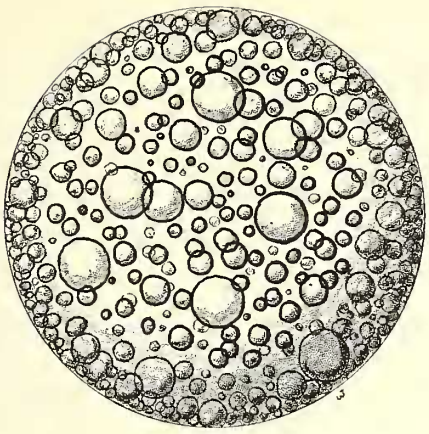
Immature eggs appear to be more opaque than mature fertilized ones. It may be that this change takes place at fertilization as it does in *Fundulus* (Oppenheimer, 1937), but in this viviparous species it is difficult to substantiate.

Stage 2. *Cleavage* (Text-fig. 2).

Cleavages may be seen only occasionally, and only in eggs preserved in formalin. The cleavage cells are very thin, broad and flat, and since they are not raised above the yolk surface to any visible extent, this stage is poorly distinguished from the previous one. Text-fig. 2 shows the cleavage stage more distinctly than it actually appears. Using a glass needle and a pair of sharpened watchmaker's forceps, the fertilization membrane can be removed from such eggs while in the saline solution, and the contents left in place. Such a membrane cannot be demonstrated around infertile ova. This fertilization membrane persists throughout the gestation period and is ruptured together with the follicle just prior to parturition.

Stage 3. *Compact Blastula* (Text-fig. 3).

This is the earliest stage which can be identified readily by gross study. The cells are small and tightly packed into a small



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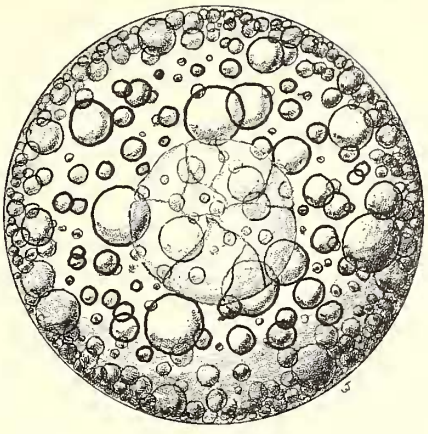

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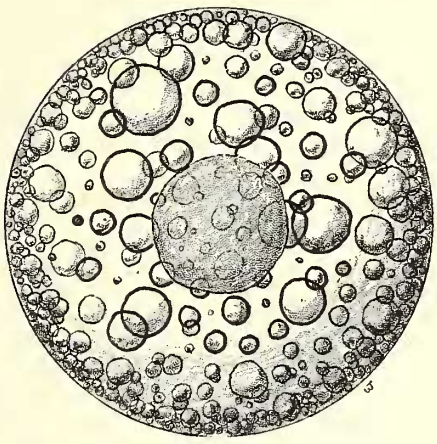

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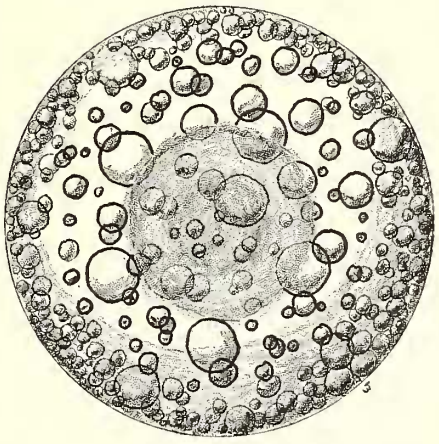

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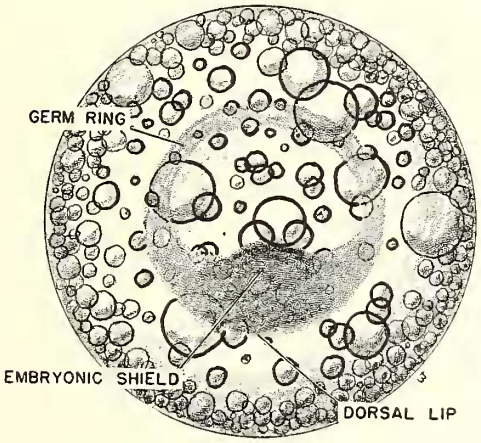

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TEXT-FIG. 1. Mature ovum. Stage 1. × 38.
TEXT-FIG. 2. Cleavage. Stage 2. Polar view. × 38.
TEXT-FIG. 3. Compact blastula. Stage 3. Polar view. × 38.
TEXT-FIG. 4. Diffuse blastula. Stage 4. Polar view. × 38.
TEXT-FIG. 5. Early germ ring gastrula. Stage 5. Polar view. × 38.

grayish protoplasmic disc, which is slightly raised above the yolk surface. A segmentation cavity has been described beneath the disc (Hopper, 1943).

Stage 4. *Diffuse Blastula* (Text-fig. 4).

Gastrulation begins at this stage with the blastodisc flattening out into a thin membrane of cells. The periphery of the blastodisc is uniformly thickened, indicating the region of proliferation and probable involution.

Stage 5. *Early Germ Ring Gastrula* (Text-fig. 5).

Gastrulation continues during stage 5 with a peripheral spreading of the blastodisc in all directions. The embryonic shield is visible as a widening and thickening of a sector of the rim of the blastodisc.

Stage 6. *Late Gastrula—Early Neurula* (Text-fig. 6).

The embryonic shield takes on an elongate form and becomes raised from the yolk surface, indicating the antero-posterior axis of the developing embryo. The notochord is present, and the anterior end of the neural keel can be seen. The nerve cord is formed from a solid core of invaginating tissue; the neurocoele appearing after invagination is completed, as seen in sectioned material. This type of neurulation is typical of teleosts.

Stage 7. *Late Neurula* (Text-fig. 7).

The germ ring at this stage is somewhat below the equator and the embryo has become further elongated. Since elongation takes place principally in the posterior portion, a region roughly corresponding to the dorsal blastopore lip of amphibian gastrulae, the anterior end of the embryo lies in much the same position as did the original embryonic shield of stage 5.

The neural keel has invaginated throughout the greater length of the embryo, and a neurocoele is present in the anterior one-fourth.

Stage 8. *Head Fold* (Text-fig. 8).

A prominent head fold is present by stage 8. The neurocoele is open for about the anterior half of the length of the embryo. The optic buds are present and attached to the short, thin stalks, and they are, at this stage, without a cavity. Two pairs of rather diffuse somites are evident, but there is considerable variation in the time of their first appearance. Somites sometimes appear as early as stage 7.

Stage 9. *Optic and Otic Vesicles;* 1.1 *mm.* (Text-fig. 9),

The head fold has now begun to elongate anteriorly. The blastopore is still a wide open structure and the caudal end has not progressed back much farther than its position in stage 8. The optic primordia now possess cavities, and are usually still attached to the prosencephalon by thin optic stalks. The brain is divided into three general regions: a narrow prosencephalon, a slightly wider mesencephalon, and a short rhombencephalon. Otic vesicles have invaginated at the level of the rhombencephalon, but are still connected to the exterior by the endolymphatic ducts. Usually, 7 pairs of somites are visible at this stage.

The pericardial sac, which develops very early, closely enfolds most of the head fold at this stage.

Stage 10. *Tail Bud;* 1.5 *mm.* (Text-fig. 10).

The optic vesicles are detached from the brain and are slightly flattened around the invaginating lens primordia. The mesencephalon and rhombencephalon have become widened and more thin walled. The otic vesicles are slightly ellipsoid and are completely cut off from the superficial ectoderm. There are ten pairs of compact somites visible. The tail bud has begun to form and extends slightly over the region of the dorsal lip of the open blastopore.

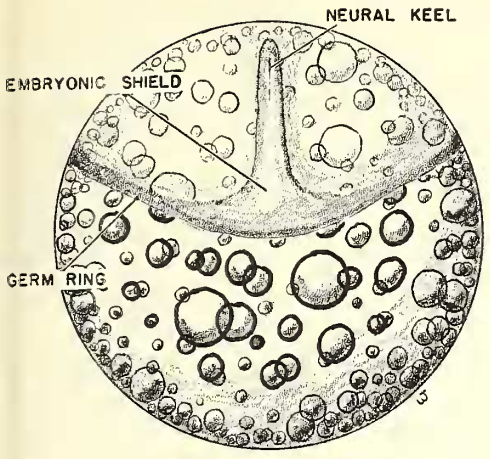
The region of the pericardial sac that is extra-embryonic is easily distinguishable, and, upon dissection, the heart can be found as a straight tube on the floor of the pericardial sac. The vascular system is apparently complete at this time, but the blood islands are never visible under gross examination. The heart exhibits no regular beat, only an occasional twitch.

Stage 11. *Pectoral Fin Buds* (Text-fig. 11).

The optic vesicles partially envelop the lens primordia. The prosencephalon shows little differentiation, but the mesencephalon has widened out considerably. Indications of neuromeres can be seen in the rhombencephalon. The entire brain possesses a thin roof, and this is especially true at the hind-brain level. In later stages, the roof of the mesencephalon becomes thickened, but that of the myelencephalon remains thin as the posterior tela chorioidea. The otic vesicles show little or no change, aside from a general growth, in this and several of the following stages. Text-fig. 11 shows the presence of the anterior fin buds.

Posteriorly, 18 to 20 small, compact somite pairs blend into a poorly differentiated region in the now prominent tail bud. It is noteworthy that, although a sizeable tail bud is present at this stage, the blastopore is open in the majority of the embryos. This is in contrast to the case in most teleosts, and even in the closely related *Fundulus*.

In the heart, the ventricular and atrial portions are distinct, and at the anterior end, the sinus venosus projects in front of the head. The heart exhibits a fairly rhyth-



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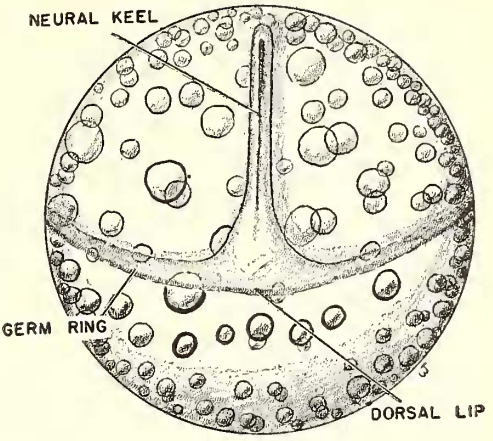

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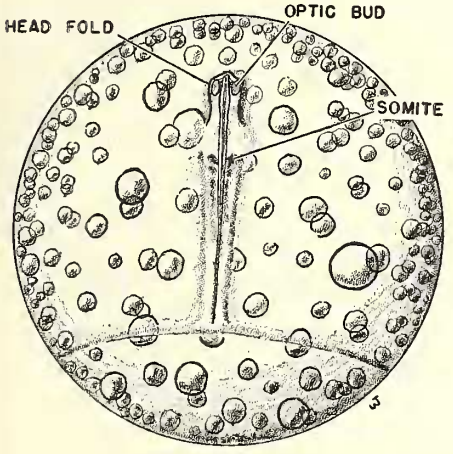

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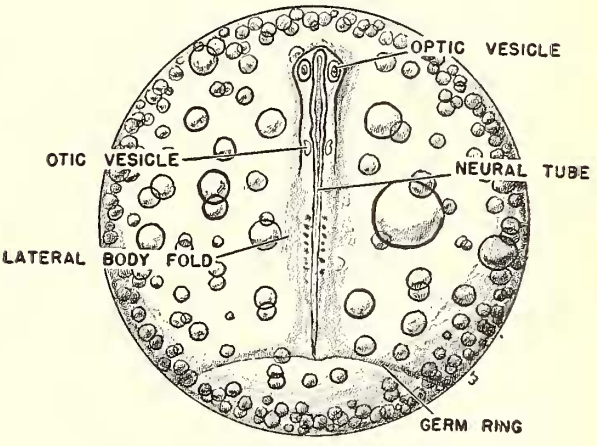

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TEXT-FIG. 6. Late gastrula–early neurula. Stage 6. Equatorial view. × 38.
TEXT-FIG. 7. Late neurula. Stage 7. Equatorial view. × 38.
TEXT-FIG. 8. Head fold. Stage 8. Dorsal aspect. × 38.
TEXT-FIG. 9. Optic and otic vesicles. Stage 9. Dorsal aspect. × 38.

mical beat at this time. The color of the blood is light pink, but barely perceptible.

Stage 12. *Regular Heart Beat;* 1.8 *mm.* (Text-figs. 12, 13).

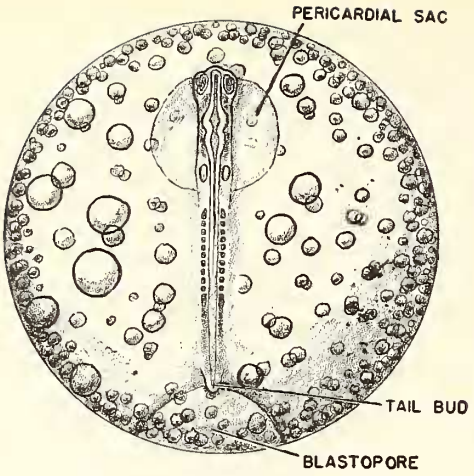
The optic cups envelop the lenses closely. Olfactory placodes are visible. The brain has undergone further development; the telencephalic region is slightly expanded; the mesencephalon has a thicker roof; the rhombencephalon is greatly widened.

The somites are more closely packed and less distinct. The vascularization of the pericardial membrane is in the form of small capillary-size vessels. The extra-embryonic circulation can be followed at this stage. The blood leaves the embryo through the ducts of Cuvier at the posterior ventral margin of the pericardial membrane, drains into the yolk portal system and the vascularized pericardial membrane, and collects at the elongated sinus venosus.

The mid-gut is broad and extends under about one-third of the embryo. The hind-gut is short, and the fore-gut, upon dissection, is shown to possess a distinct first pharyngeal pouch and a corresponding visceral furrow.

Stage 13. *Early Retinal Pigment;* 2.1 *mm.* (Text-figs. 14, 15).

Olfactory pits are distinct. Pigment can be seen in the retina as a thin gray band. The brain and the head are further enlarged. The pericardial sac has increased to its maximal size. In the future stages



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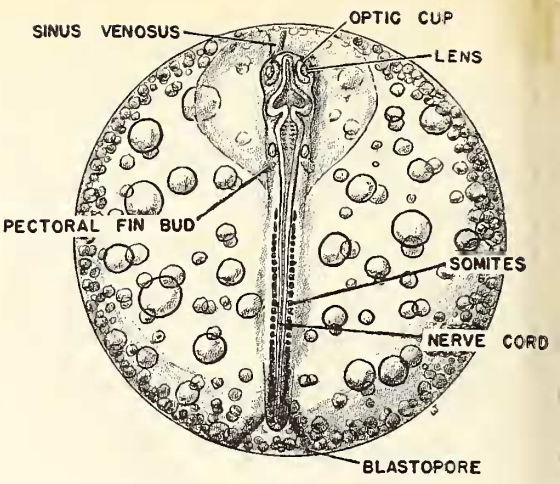

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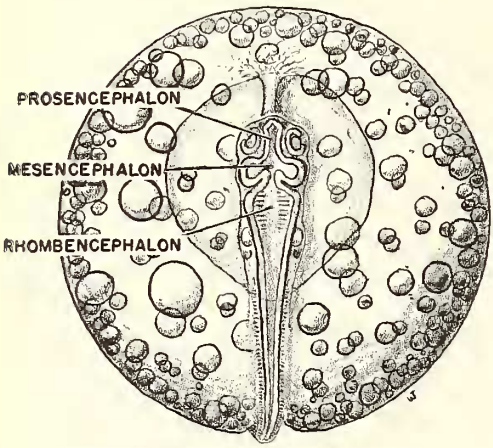

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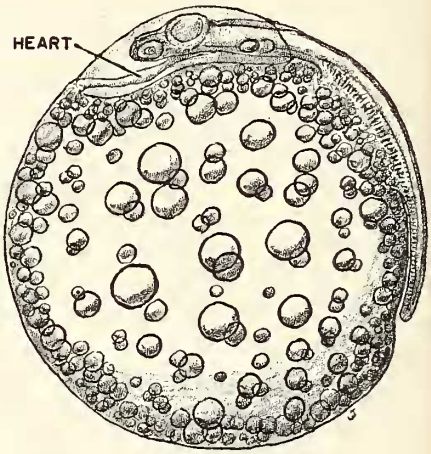

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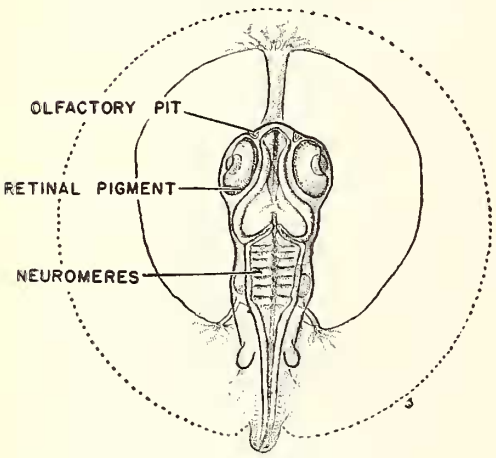

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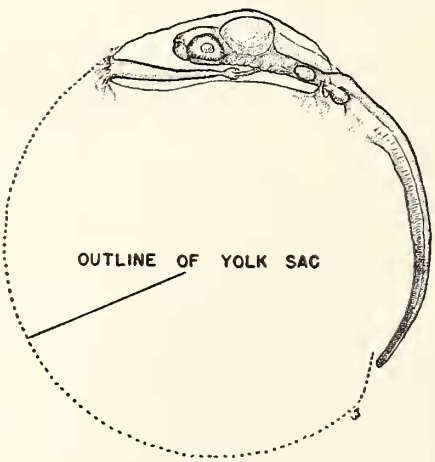

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TEXT-FIG. 10. Tail bud; 1.5 mm. total length. Stage 10. Dorsal aspect. × 38.
TEXT-FIG. 11. Pectoral fin buds. Stage 11. Dorsal aspect. × 38.
TEXT-FIG. 12. Regular heart beat; 1.8 mm. Stage 12. Dorsal aspect. × 38.
TEXT-FIG. 13. Stage 12. Lateral aspect. × 38.
TEXT-FIG. 14. Early retinal pigment; 2.1 mm. Stage 13. Dorsal aspect. × 38.
TEXT-FIG. 15. Stage 13. Lateral aspect. × 38.

the head enlarges to fill the serosa-like cavity and sinks down into the yolk mass. In side view, the stomodaeum, five gill clefts and the sixth furrow can be seen.

Stage 14. *Early Motility*; 2.8 *mm.* (Text-figs. 16, 17).

The head is expanded to almost 0.5 mm. across the mesencephalon. The eyes exhibit more pigment and are pushed forward by the expanding mesencephalon. The latter possesses a thickened roof where the optic lobes are developing. The telencephalon has a somewhat rhomboidal-shaped cavity and the diencephalon is small and hardly distinct; this is typical of the teleosts. Both the metencephalon, which is poorly defined, and the myelencephalon have thin roofs. The neuromeres are still visible in the latter.

The heart possesses a long sinus venosus and a narrow atrium that has been twisted to the left of the thick-walled ventricle. The blood vessels of the pericardial membrane are enlarged to a size equal to almost one-half the diameter of the ducts of Cuvier.

The anterior fin-buds are club-shaped and rounded. The somites have taken the form of myotomes, and, when the living embryo is removed from its membranes, the posterior portion exhibits a slow twitching motion. The tail is conical and acuminate.

All six gill slits are distinct and open at this stage. The mid-gut is narrowed toward the posterior portion of the embryo, and fore-gut is an undifferentiated tube.

Stage 15. *Otoliths in Ear Vesicles*; *First Extra-ocular Melanophores;* 3.1 *mm.* (Text-figs. 18, 19).

In this stage the telencephalic vesicles are beginning to show as lateral bulges. The diencephalon is shorter than the telencephalon and less distinct. The optic lobes possess a solid roof. The metencephalon is more distinct and thickened, and the myelencephalon is somewhat narrowed.

The eye pigment has become considerably darker and some iridiophores are present. The pupil is ellipsoidal. The olfactory bulbs have completely invaginated. The otic vesicles are enlarged and three crystal-like otoliths are present in each.

The fin buds are laterally flattened. The caudal tip of the notochord is slightly upturned and the tail tip is laterally compressed, exhibiting a rudimentary sign of a heterocercal type of tail structure.

A few stellate melanophores are usually found in the connective tissue above the mid-dorsal, posterior region of the mesencephalon. This is the first indication of extra-ocular melanophores.

The gut is completely separate from the yolk and the anterior intestinal portion is twisted into two coils. The posterior portion is straight and ends in a somewhat long post-anal region. The gill slits, except the first, are beginning to sink into a common cervical sinus, the forerunner of the opercular cavity.

Stage 16. *Fin Rays;* 3.2 *mm.*

First indications of fin rays in the caudal and pectoral fins are present. Melanophores are spreading to the myelencephalon region.

Stage 17. *Anal and Ventral Fins;* 3.4 *mm.*

Anal fin and the skeletal elements of the ventral fins are beginning to appear. Smaller, dot-like, melanophores appear on the lateral body folds. Head is further enlarged and fills the entire pericardial membrane tightly. The operculum is formed at this time.

Stage 18. *Dorsal Fin;* 3.7 *mm.*

Primordium of dorsal fin becomes visible, but there are no skeletal elements within it. Melanophores have spread over the entire mid- and hind-brain regions. Embryos at this stage are capable of swimming about, although the yolk sac prevents them from rising from the substrate.

Stage 19. *Eyes and Mouth Mobile;* 3.9 *mm.*

Through the enveloping pericardial membranes, the eyes may be seen to move and the mouth to open. The operculum is functional here. Fascial and peritoneal melanophores appear as small black dots.

Stage 20. *Pericardial Sac Splitting;* 4.2 *mm.*

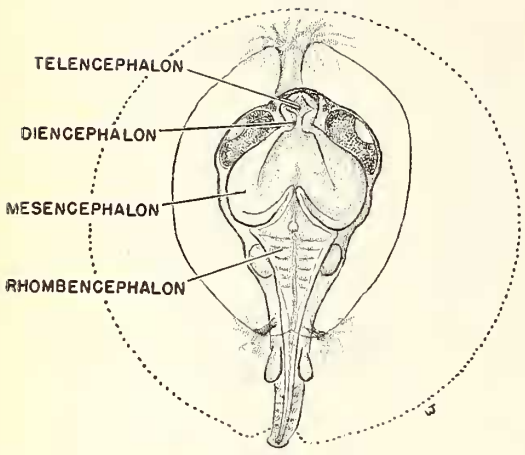
The pericardial extra-embryonic membrane begins to split down the dorsal midline, starting at the anterior margin just above the sinus venosus. (This is the first step in the formation of the "neck strap," described by Turner (1940a) in many viviparous cyprinodonts).

Stage 21. *Mouth Protruding;* 4.6 *mm.*

The pericardial sac has split open as far as the anterior margin of the eye, allowing the mouth to protrude. The peritoneal melanophores are more numerous and small fascial melanophores are concentrated around the notochord. Stellate cutaneous melanophores are very sparsely scattered over the entire embryo, and many are concentrated in the mid- and hind-brain regions.

Stage 22. *Broad "Neck Strap;"* 5.1 *mm.* (Text-fig. 20).

The pericardial membrane has split as far back as the posterior third of the eye, exhibiting a broad, vascularized "neck strap." The appearance of sclerotomes is here accentuated by concentrations of small



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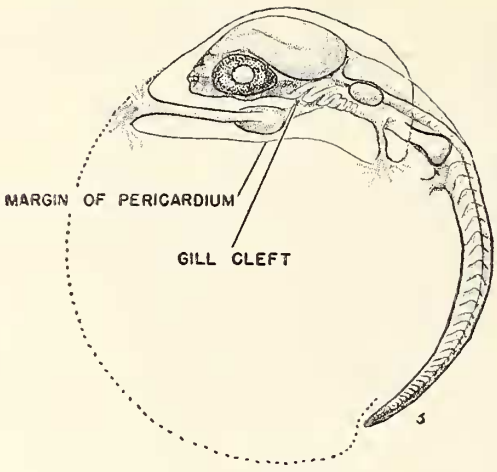

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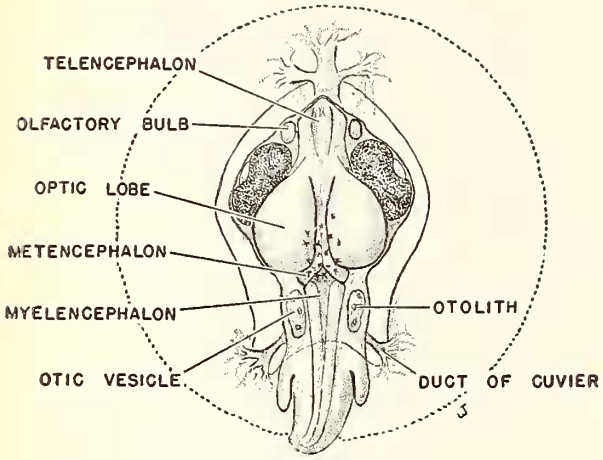

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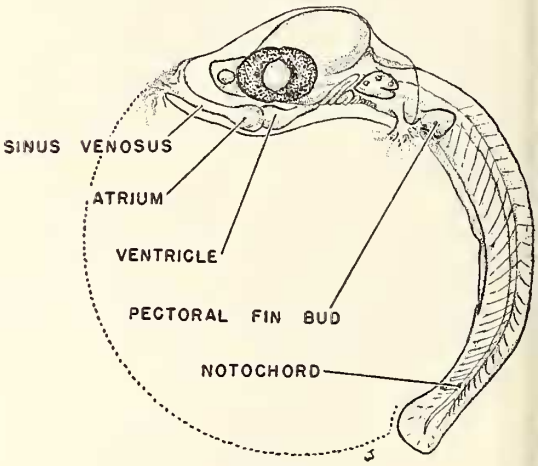

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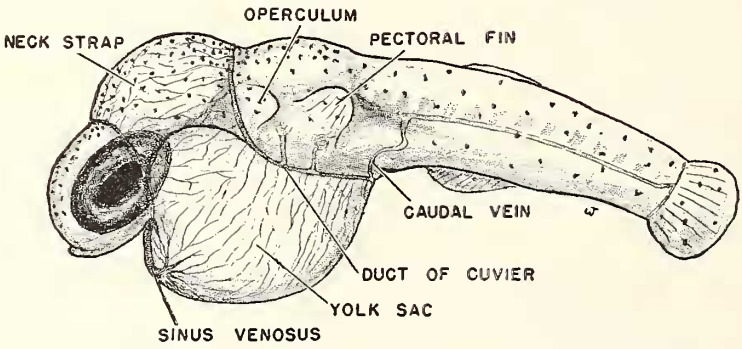

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TEXT-FIG. 16. Early motility; 2.8 mm. Stage 14. Dorsal aspect. × 38.
TEXT-FIG. 17. Stage 14. Lateral aspect. × 38.
TEXT-FIG. 18. Otoliths; Extra-ocular melanophores; 3.1 mm. Stage 15. Dorsal aspect. × 38.
TEXT-FIG. 19. Stage 15. Lateral aspect. × 38.
TEXT-FIG. 20. Broad "neck strap"; 5.1 mm. Stage 22. Lateral aspect. × 38.

melanophores in the fascial tissue around them. Cutaneous melanophores are more numerous, sometimes present in the caudal fin rays.

Stage 23. *Fin Rays in Dorsal Fin;* 6.1 *mm.*

The "neck strap" (pericardial membrane) has been reduced to about one-half the width of the eye, and is situated back of the posterior margin of the eye. Fin rays begin to appear in the dorsal fin. Large cutaneous melanophores are thickly scattered over the entire embryo. The yolk sac begins to show a rapid reduction in size, measuring 1 mm. in diameter. It is noteworthy that the yolk sac begins to involute at about the same time that the pericardial membranes are in the process of accelerated regression.

Embryos at this stage, if removed from their mothers, will feed readily on small *Daphnia.*

Stage 24. *"Neck Strap" Breaking Down;* 6.5 *mm.*

The "neck strap" may be completely broken down at this stage, but it is sometimes present as a narrow band of tissue. The general shape of the embryo is determined by the condition of the "neck strap," the cephalic flexure straightening as the head lifts up into the main body axis. The melanophores in the dorsal head region are stellate and more closely packed.

Stage 25. *Pre-Parturition;* 6.9 *mm.*

The extra-embryonic membranes and the yolk flanges are absent. The yolk has been reduced to a mean diameter of .8 mm. No trace of the adult color pattern is yet visible, there being only a general increase in the number of melanophores on the peripheral areas. This is true even in embryos of Culture Nos. 187 and 195, where the adult pattern (induced by the gene *Sp* for spotting and *St* for stippling) is composed of large masses of macromelanophores and micromelanophores. Nor can these two types of melanophores be distinguished.

Stage 26. *One Hour after Birth;* 7.9 *mm.*

Birth activity begins with a rupture of the fertilization and follicle membranes by the violent movements of the embryos. The embryos break into the ovarian sac and then one by one they are extruded through the oviduct into the water.

In earlier stages, the heart extends forward from the conus, and the sinus venosus lies directly beneath the tip of the head. As the yolk mass becomes reduced, the heart pivots on the conus and the yolk sac portal system shrinks until the ducts of Cuvier drain directly into the sinus venosus, which eventually moves into place posterior to the conus.

Growth proceeds rapidly and within 24 hours after birth the young fry reach an average length of 8.7 mm.

Rate of Development.

In order to obtain some estimation of the developmental rate in *Platypoecilus maculatus,* records were kept on the number of embryos and their stages found in each timed gravid female. The morphological age of the embryos was determined by comparing each with the twenty-five established graded stages.

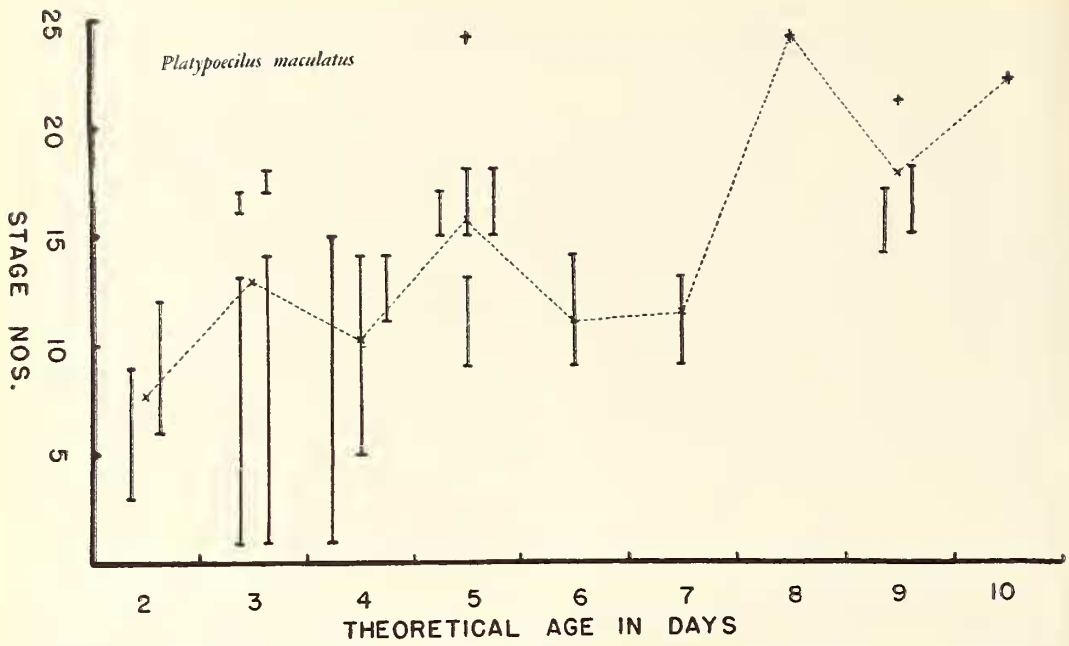
The following terms are used in this section: *Theoretical age* is the value determined for the entire embryonic brood from the date of the previous brood, less the seven day interval (as determined by Hopper, 1943). *Morphological age* for each embryo is established by comparison with the graded series of stages. *Chronological age* represents the actual developmental rate for each stage.

The theoretical age of all the members of a brood was determined by recording the date of birth of a previous brood. This is based upon the fact that fertilization of a successive complement of eggs within a gravid female takes place on about the seventh day after the birth of its previous brood (Hopper, 1943). Theoretically then, the embryos carried by a gravid female, which had dropped a brood eight days previously, are 24 hours old.

This theoretical age value, it must be noted, is only an approximation, since maturation and fertilization of a complement of eggs is spread apparently over a period of two or three days. The seven day interval, as determined by Hopper (1943), has been found to be only an average time lapse. The estimation of the true chronological age may be determined by comparing the theoretical and the morphological age values.

A reliable estimation of the theoretical age was obtained by study of those broods from fully matured females which contained 25 or more embryos, and which had given birth to at least two previous broods at an interval of approximately 28 days. Only 21 out of 55 females examined had these qualifications. Data on many young females were found to be unreliable since many of them had run highly irregular reproductive cycles, varying from 35 to 90 days between broods; and a large percentage of their embryos were dead or abnormal. For these purposes, too, data on exceptionally small embryonic broods (those containing less than 10 embryos) were not considered.

The chart (Text-fig. 21) summarizes the data on 21 embryonic broods plotted in the following manner: Each vertical bar



TEXT-FIG. 21. Chart showing age variations in 21 embryonic broods. Each vertical bar represents an entire brood removed from a female. Dotted line connects the mean morphological ages for each theoretical age group. (See detailed description in text.)

represents all the members of one entire brood carried by a single gravid female platyfish. The length of each vertical bar, projected on the ordinate, shows the range of morphological ages found in each embryonic brood. In some cases, especially during the later portion of the gestation period, the embryos are all of a single morphological age; these are represented by plus (+) signs.

The embryos are divided into theoretical age groups according to the number of days that have elapsed since the birth of the previous brood (less the seven day interval) and are arranged along the abscissa. Usually there is more than one brood in each age group.

The mean morphological ages for all the embryos of each theoretical age group are also plotted on the chart, and these values are connected by the dotted line.

From the chart, it may be seen that there are two kinds of variations. First, there is the wide range of morphological stages among the embryos found within any one gravid female; and second, the variations of the average morphological age of a brood with respect to its theoretical age.

The greater apparent spread of morphological stages in the earlier broods may be attributed to the unequal time lapse between stages distinguished on the basis of morphology alone.

Using the information described previously on the reproductive cycle of the platyfish, it was thought that not only a graded series of morphological stages but also a chronological series could be obtained. On the basis of these data, some estimations of the time of development of each stage could have been made. However, the variation, as demonstrated by the chart, proved to be so great that an estimation of the true chronological age was impossible.

DISCUSSION.

Adaptations for Viviparity in the Cyprinodonts.

The family Poeciliidae is one of several belonging to the teleostean order Cyprinodontes (killifishes or top-minnows, Hubbs, 1924). The type family, Cyprinodontidae, includes many oviparous forms such as the common coastal killifish (*Fundulus heteroclitus*) and the Japanese medaka (*Oryzias latipes*). The embryonic stages for these two fishes have been described by Oppenheimer (1937) and Rugh (1941), respectively.

The Cyprinodontidae are characterized by the possession of macrolecithal eggs, and an enlarged pericardial sac in the embryo, which is vascularized and serves, apparently, as an accessory respiratory organ.

The family Poeciliidae, which includes *Platypoecilus maculatus*, is composed of several viviparous species commonly used in home aquaria, such as the guppy (*Lebistes*

reticulatus), and the swordtail (*Xiphophorus hellerii*). In these viviparous forms, fertilization and development of the embryos take place within the ovarian follicle. The poeciliids also possess a greatly expanded pericardial sac which forms an enveloping extra-embryonic membrane, (see discussion on development of pericardium). Other poeciliids have evolved certain morphological adaptations for viviparity, which have been described by Turner (1940a). For instance, *Heterandria* has a highly developed extra-embryonic pericardium and a reduced yolk, and *Poeciliopsis* and *Aulophallus* have, in addition, a highly expanded coelom and a follicular pseudoplacenta. Superfoetation is exhibited to a high degree in these three.

The members of the Goodeidae are viviparous and their embryos develop within ovarian follicles. The size of the goodeid pericardial sac may vary but it never reaches the large proportions of that of the poeciliid (according to Turner, 1940b). The goodeid yolk sac is small and transient. Vascularized outgrowths of the cloacal lips (trophotaeniae) are present, and apparently these structures serve as organs for absorbing oxygen and nutriments from the maternal circulation. *Goodea* possesses a small rosette-shaped trophotaenia, and *Characodon* possesses two elongate trophotaeniae. These structures are larger in *Girardinus* and *Zoögeneticus*. In *Lermichthys*, the climax is reached with an early and extensive development of the trophotaeniae. These structures have been used by Hubbs & Turner (1937) as a basis for a taxonomic revision of the group.

In the family Anablepidae (according to Turner, 1940c) there is an enlarged pericardial sac and a highly developed follicular pseudoplacenta. The transient yolk sac is replaced early in the gestation period by an elongate and coiled mid-gut in *Anableps anableps* or a distended posterior intestine in *A. dowei*.

Turner (1940d) has also shown that the fishes of the family Jenynseidae differ from all of the above forms in that, although fertilization occurs in the follicle, most of the embryonic development takes place in the ovarian lumen. Here the yolk sac and the pericardial sac are ephemeral structures; the embryo absorbs nourishment through a flap of the ovarian tissue which enters the pharyngeal cavity of the embryo through one of its opercular clefts.

Although the adaptations described above are presumed to be for the purpose of obtaining nourishment from the maternal circulation, it has not been shown experimentally just what functions the various structures serve. Does absorption, for instance, take place through the walls of the ovarian blood vessels or directly from the coelomic fluid? Scrimshaw (1944, 1945) has shown that many of these forms are truly viviparous, not ovoviviparous, by recording the dry weights of embryos at various stages of gestation. Species of *Xiphoporus*, *Lebistes*, *Gambusia* and many of the Goodeidae showed no loss in dry weight throughout the gestation period, indicating that there is a continuous replacement of nutrient materials and a draining of metabolic wastes. *Heterandria*, a known superfoetacious form, exhibited a notable increase in weight, indicating not only replacement but addition of growth-promoting substances.

In Stage 23 of *Platypoecilus* the yolk sac begins to involute coincidently with the accelerated regression of the pericardial membranes. This may indicate a change in the mechanism by which the embryo obtains nutrient materials. In early development it presumably received them through the medium of the highly vascularized pericardial membranes. As the membranes became radically reduced after Stage 22, the embryo may obtain from the yolk the bulk of the nourishment it requires to complete its development.

Embryological Stages of Platypoecilus *and* Fundulus *Compared.*

The unfertilized mature egg of *Fundulus* differs from that of the platyfish in the possession of a thick tertiary membrane, i.e. the chorion, deposited by maternal tissues. The fat globules are clumped in the center of the egg, instead of being spread on the periphery as in the platyfish. Oppenheimer (1937) notes that the number and size of the fat globules are characteristic for each female; this is also true for the platyfish.

The cleavage cells of *Fundulus* eggs are globose and distinct, whereas those of the platyfish are thin, broad, and flat. The "expanding blastula" (stage 11 of *Fundulus*) is very similar to stage 4 of *Platypoecilus*. Stage 13 (*Fundulus*) is quite similar to stage 7 (platyfish). From here on, the differences become more marked.

In *Fundulus*, the blastopore is closed usually before somites or optic vesicles develop, but in *Platypoecilus* the blastopore remains open even up to tail bud formation (see Text-fig. 11).

Platypoecilus shows the development of cavities in the optic vesicles simultaneously with the invagination of the otic vesicles, at stage 9. In *Fundulus*, the optic vesicles are formed at stage 17, and otic vesicles are not present until the 20-25 somite stage (stage 20). However, stage 12 of *Platypoecilus* and stage 20 of *Fundulus* are superficially very similar.

Otoliths are first visible in *Platypoecilus* by stage 15, and by stage 23 of *Fundulus*. However, the general shape of the *Fundulus*

embryo at this stage closely resembles stage 13 of *Platypoecilus*.

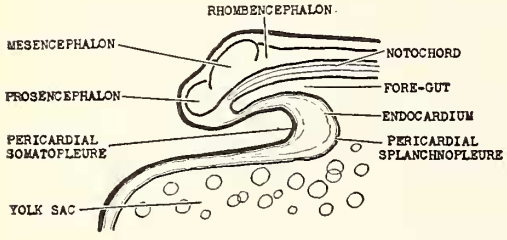
In the platyfish, the first evidence of pigment is in the retina (stage 13), and the first extra-ocular melanophores appear in stage 15. In *Fundulus*, melanophores are first visible over the yolk in stage 19, simultaneously with the formation of the lens and olfactory pit, and retinal pigment first appears in stage 27 where cutaneous melanophores are already present.

The most striking difference between the two above forms is the presence of an enveloping pericardial sac in *Platypoecilus* which forms the extra-embryonic membranes, described below.

Development of Extra-embryonic Pericardium.

In the oviparous cyprinodonts, i. e. *Fundulus*, the pericardial sac develops early in ontogeny as an outgrowth of the somatopleure just ventral to the heart primordium (Turner, 1940a). It balloons out, carrying with it the posterior end of the network of blood vessels anastomosing to form the heart. By the time the heart has differentiated, the pericardium has expanded anteriorly beyond the head fold. The heart, then, is in a reversed position at this stage, the sinus venosus being anterior and the conus posterior (Text-figs. 22, 23), and its entire length is suspended over the floor of the pericardium, i. e. yolk sac splanchnopleure. The head overlays this expanded pericardium and dips down into it. In *Fundulus* and *Oryzias*, the pericardial sac becomes vascularized, and serves, apparently, as an accessory respiratory organ.

In the viviparous Poeciliidae, the development of this sac is carried further. Turner (1940a), in describing the condition in *Heterandria*, shows that the pericardial sac expands over the anterior one-third of the embryo and enfolds it in a double-layered sac. The inner layer is thin and closely applied to the head, and the outer layer is vascularized and balloons out over the embryo. For the other, less specialized poeciliids, Turner describes a structure which he calls the "neck strap," that extends as a belt over the posterior head region of the embryo. He reports the presence of such a structure in species of *Platypoecilus*, *Xiphophorus*, *Gambusia*, *Lebistes* and other related genera. According to Turner, this "neck strap" originates from the yolk sac membrane and the yolk flanges. Our observa-



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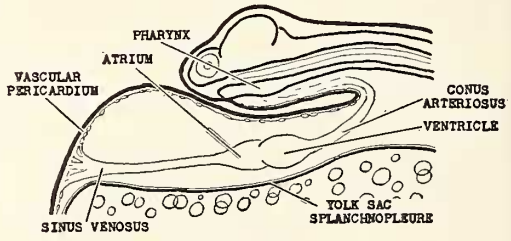

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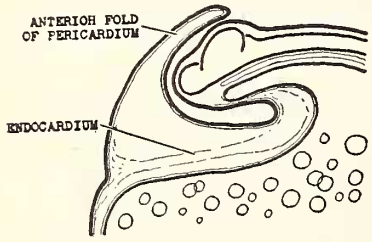

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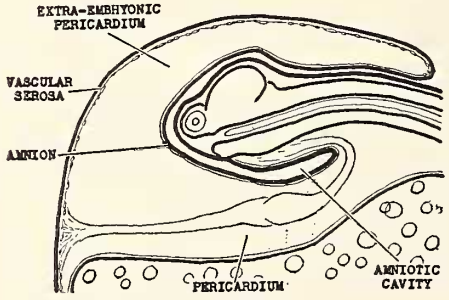

25.

TEXT-FIG. 22. Diagrammatic sagittal section of *Fundulus* embryo before development of pericardial sac.

TEXT-FIG. 23. Diagrammatic sagittal section of *Fundulus* embryo at maximum development of pericardial sac.

TEXT-FIG. 24. Diagrammatic sagittal section of *Platypoecilus* embryo at beginning of formation of extra-embryonic pericardium.

TEXT-FIG. 25. Diagrammatic sagittal section of *Platypoecilus* embryo at maximum development of the pericardial amnion and pericardial serosa.

tions indicate that the pericardial sac in *Platypoecilus* develops just as it does in *Heterandria*. During development of the embryo the pericardial sac is broken down by the enlarging head and at some stages an ephemeral "neck strap" appears (Text-fig. 20). A preliminary examination of the "neck strap" in early embryos of *Lebistes* and *Xiphophorus* shows the same changes. The pericardial sacs of all these fishes reach the same proportions in development as that of *Heterandria*. Turner's report on the platyfish, guppy, swordtail and other poeciliids, was apparently based on somewhat older embryos. An examination of comparable, early stages of the other genera mentioned by Turner may indicate a common developmental history of the pericardial sac in the poeciliids.

The pericardium of *Platypoecilus* begins its development as it does in *Fundulus*, but continues on to envelop the anterior one-fourth of the embryo in a two-layered sac (Text-figs. 24, 25). The mechanism of this overgrowth by the somatopleure is basically the same as the formation of the amnion and serosa of reptiles and birds. The development of extra-embryonic membranes in the Poeciliidae in a manner very similar to their derivation in the amniotes is an interesting example of parallelism in evolution. In both groups, the membranes are developed from somatopleure and may be considered homologous. Therefore, the terms "amnion" and "serosa" may properly be applied to the poeciliid membranes. However, "pericardial amnion" and "pericardial serosa" are suggested for the membranes of this group of fishes in order to avoid any possible confusion with the homologous membranes in the amniotes. Turner (1940a) had previously called the amnion of viviparous cyprinodonts "the pseudoamnion" and applied the term of "pseudochorion" to the cyprinodont serosa.

The term "chorion," as applied to avian and mammalian embryology, is synonymous with "serosa," for it is an extra-embryonic membrane derived from the somatopleure. However, the "chorion" in fishes is a tertiary membrane deposited around the eggs while in the ovary of oviparous species by the follicle cells. The use of the term "pseudochorion" for the extra-embryonic membrane in poeciliids might prove confusing, and it is felt that "pericardial serosa" should be used here, since there are no maternal tissues involved.

Development of Melanophores.

The earliest visible melanophores appeared during stage 13 and were found within the retina. The first visible extraocular melanophores appeared during stage 15 above the mid-dorsal, posterior region of the mesencephalon. Gordon (1931a) had previously figured a 2 mm. stippled (wild-colored) platyfish showing that the ocular pigmentation was completed at this stage of development while the pigmentation over the brain had barely begun. In the brain region, the pigmentation was represented by about 15 irregular, stellate melanophores whose dendritic processes showed a series of anastomoses. In the 3.5 mm. embryos of the same genetic strain, the melanophores over the brain area had increased in numbers, in size and in melanin content; anastomoses of the irregular dendritic processes persisted. In the 4.5 mm. embryos, the long dendritic processes were reduced and the pigmented cells rounded up, taking the shape of discrete micromelanophores of which there were about 50 over the brain area. During the latter periods cutaneous melanophores gradually appeared over the rest of the body. (The measurements used by Gordon are "standard length," i. e. the tail fin length is not included.)

Finally, in the stage just prior to birth, in the 5.5 mm. embryo the number of definitive micromelanophores increased to over 250 in the brain area. Apparently, these pigment cells are perineural, rather than cutaneous melanophores, although they are morphologically similar, for they were lodged in the meninges around the brain. Owing to the thinness and transparency of the integument over the brain, the pigmentation of this region appears to be that of the skin, whereas it is that of the meninges.

At the time micromelanophore development was for the most part completed over the brain area, the condition of the pigment cells in the integument over the rest of the body was in the early or stellate stage. No evidence was offered, one way or the other, to show whether the "stellate" pigmented cells were transformed into definite micromelanophores or whether micromelanophores replaced the early appearing dendritic pigment cells.

In the present observations on the developmental stages, no recognizable macromelanophores could be detected in pure *Platypoecilus maculatus* embryos genetically constituted to have them. Gordon & Flathman (1943) were able to identify the larger melanophores in platyfish-swordtail hybrid embryos. And Gordon & Smith (1938) showed that in some back-cross hybrids (*Platypoecilus maculatus-Xiphophorus hellerii* × *Xiphophorus hellerii*) the macromelanophore growth had proceeded so rapidly in the melanotic embryos that these cells had invaded the subcutaneous areas where the destruction of muscle tissue was begun.

DuShane (1943) has shown experimentally that melanophores in amphibia arise

from the neural crest cells in early development and similar proof is available for the origin of melanophores in birds. DuShane credits Borcea (1910) with being the first to suggest that pigment cells have a neural crest origin and Borcea based this suggestion on his observations of the developing embryos of the pipefish, *Belone*. However, this conclusion has not as yet been proved experimentally in fishes.

The final statement concerning the point of embryonic origin of the melanophores in the platyfish must await the development of successful techniques for extra-ovarian culture of the early growth stages.

Summary.

1. The female platyfish, *Platypoecilus maculatus*, once inseminated, will produce as many as four or more successive broods at 28-day intervals without further contact with males, the sperm being stored within the folds of the oviduct. Fertilization of the ova usually takes place seven days after the release of the previous brood. Broods were removed from females at various intervals after the birth of a brood, and the developmental stages were studied, described and classified.

2. There was found a considerable variation in the developmental stages among the embryos of a given gravid platyfish. Since all the embryos of a brood are born within a space of an hour, the belated embryos either die or catch up to the age of the brood as a whole. Considerable variation was found in the relation of the average morphological age of the embryonic broods with respect to their theoretical age (i. e. as determined by the date of birth of the previous brood).

3. The platyfish is truly viviparous. In early development, the pericardial membrane is very extensive and is presumed to be the main mechanism through which the embryo obtains nourishment from the mother. Later, the pericardial membrane regresses and it is assumed that the embryo then relies on the yolk as its main food source. Other mechanisms for attainment of food and oxygen are developed in other viviparous cyprinodonts.

4. *Platypoecilus* differs from *Fundulus* chiefly in the development of extra-embryonic membranes. In *Fundulus*, the highly vascularized pericardial sac serves as an accessory respiratory organ. In *Platypoecilus*, the development of this organ is carried further, enveloping one-fourth of the embryo, and it presumably brings nutrition to the embryo.

5. In the platyfish, the pericardial membranes, i. e. pericardial amnion and pericardial serosa, envelop the entire anterior portion of the embryo during their maximal development. During the process of regression of these membranes, a temporary "neck strap" structure is formed. The mechanism of overgrowth of the platyfish membranes is basically the same as the formation of the amnion and serosa of reptiles and birds.

6. The development and distribution of melanophores in platyfish embryos is described. No differences could be noted between micro- and macromelanophores. Initial pigment distribution is the same in all the pattern strains of *P. maculatus* that were studied.

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